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BY

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INTRODUCTION AND RATIONALE

From 1951 to 1956, scientists of the Pacific Oceanic Fishery Investigations (now the Honolulu Laboratory of the Southwest Fisheries Center, National Marine Fisheries Service) investigated the reactions of captive and wild tuna to chemical, visual, auditory, and electrical stimuli (described in Tester 1959). In three separate investigations of tuna responses to chemical stimuli (van Weel 1952; Tester et al. 1954; Tester et al., 1955) captive tuna (Euthynnus affinis and Thunnus albacares) responded to the aqueous and alcohol extracts of tuna flesh, and some other fish but little response to other odors, such as amino acids, proteins, and aromatics. Fish sometimes responded slightly better to the extracts of the food that they were being fed at the time. Field tests of a tuna flesh extract, with a pole-and-line tuna boat with live bait, yielded variable results, and it was concluded that the extracts could not reliably hold the tuna at the stern of the boat. However, these tests were sometimes confused by the presence of live baitfish remaining in the water at the time of the extract tests. In addition, edible and inedible lures (which were taken by the captive tuna if soaked in extract and made visually attractive) were not successful in sea tests.

John E. Bardach and Reginald Gooding, in 1967, conducted experiments at the Kewalo Research Facility of the Honolulu Laboratory on captive kawakawa, E. affinis, observing the responses of the tuna to the rinses of food items. The tuna showed increased swimming speeds, a tendency to swim figure eight's around the odor source, and definite localizing behavior in some tests (cited in Bardach and Atema 1971). A high chemical sensitivity was demonstrated.

In 1977 on a Marine Mammal Commission contract (NOAA 03-6-208-35385), Jelle Atema, Kim Holland, and I conducted a series of experiments testing prey rinses on captive yellowfin tuna (Atema et al. 1980). Videotape-documented observations were made of the chemical sensitivity and behavior of the fishes with the purpose of determining the feasibility of an olfactory lure for these fish.

It was demonstrated that the yellowfin tuna are highly sensitive to the aqueous rinses of whole, undamaged prey and their proteinaceous fractions. The yellowfin tuna also responded to certain amino acid solutions at estimated concentrations as low as 10^{-11} M but the fish were even more sensitive to the whole prey rinses and did show some food conditioning of responses. The responses were highly reminiscent of the exploratory feeding behavior seen in other fish (Steven 1959; Haynes et al. 1967; Holland 1978).

The previous studies imply that olfaction must have important functions in tuna feeding behavior. Olfactory attractants have proven effective at attracting other fish to the sources of odor (Hobson 1963; Kleerekoper and Gruber 1975; Sutterlin 1975; Pawson 1977, 1978) thus there is reason to believe that an effective olfactory attractant could be developed for tuna.

This project reported here was designed to investigate the feasibility of using an olfactory attractant to modify yellowfin tuna behavior to separate tuna and the porpoise in a purse seine net. There was a possibility that this separation could be achieved because we had demonstrated that yellowfin tuna show a behavioral response (Atema et al. 1980) to the odors of prey foods (Ikehara et al. 1978) and porpoise cannot because they lack organs of smell and the necessary nervous connections for it (Jansen and Jansen 1969). This differential sensitivity could be exploited to affect the behavior of the tuna and not the porpoise. If we could develop a potent enough attractant, we could use it to "lure" the tuna school away from the porpoise school in the net to facilitate the backdown release of porpoise and reduce incidental porpoise mortality. An olfactory attractant would also be useful in alternative fishing methods, schoolfish fishing, or aggregation object fishing.

The research reported here was planned in three main phases : 1) the continued in-tank testing of odors on captive yellowfin tuna in Honolulu, Hawaii; potential attractants were tested and formulated so that a practical olfactory attractant was ready for limited testing in Phase 2, 2) preliminary field testing of the olfactory attractant were conducted in Hawaiian waters on local fishing boats to develop the most effective combination of odors and dispensing mechanisms, and 3) field testing of the olfactory attractant on board the dedicated vessel MV Queen Mary, a purse seine vessel chartered by the United States Tuna Foundation to conduct research cruises during 1978 to study the tuna-porpoise problem. This phase included the production of enough olfactory attractant to field test under actual fishing conditions, the execution of these tests and the evaluation of the attractant for the purpose of separating tuna and porpoise.

In August 1978, at the end of the dedicated vessel cruise, the project was extended for an additional 7 mo and additional funds added for an investigation into the feasibility of using electrophysiological bioassays of tuna for testing of odors.

PHASE 1 - IN-TANK TESTS

Introduction

The work in Phase 1 was designed to develop an effective olfactory attractant for testing at sea. The attractant had to be strong enough to cause significant localization behavior by the fish, to be practical for field use, and inexpensive enough to produce in quantity for testing.

Since many of the olfactory studies implicate low molecular weight proteins as the active constituents of food odors, most of the odors tested consisted of protein fractions of amino acid mixtures.

Methods

The methods used here are similar to those developed by Atema et al. (1980). Therefore, the methods are given briefly where they differ from the previous study.

To determine whether odor attractants could be used to direct tuna school behavior, a larger tank more representative of the environment of the purse seine net was necessary. The large, oval concrete tank (16.8 m x 12.2 m x 2.6 m deep) at the Kewalo Research Facility was chosen for the main experimental tank and a small circular tank (7.1 m dia. x 1.2 m) was used as a supplementary tank.

In both tanks, an odor delivery system was designed and constructed to establish a plume of odor in the tank without introducing any extraneous cues. A constant-head, aerated reservoir inside the tower provided seawater for the delivery system from the same source as the tank water. The basal flow rate of the introduction line was 3.4 liter/min to the large tank and 4.2 liter/min to the small tank. The addition of odor produced a negligible increase in flow rate. There was a choice of two input stations for the large tank and three for the small tank. With this system, no rheotactic, visual (because the odors were usually colorless), or auditory cues were evident and the inputs were randomized. With fluorescein sodium dye, in separate tests, the odor plume was visualized and documented. Thus, its dispersion and course was known over time.

A school of yellowfin tuna was established in the large tank with a great deal of initial difficulty. Until the fish were acclimated and feeding, the mortality was very high. Once a stable school was established, some yellowfin tuna were transferred into the small tank. The main school was maintained with an occasional delivery of new fish. The new fish acclimated more rapidly due to the presence of an already feeding school.

The fish ranged from 1 to 4 kg when caught and immediately underwent training to eat a variety of dead foods. Frozen surf smelt, Hypomesus pretiosus, was the primary food, supplemented by nehu, Stolephorus purpureus, northern anchory, Engraulis mordax; and later, squid, Loligo opalescens, dolphinfish, Coryphaena hippurus, and tuna flesh. The fish were usually fed once daily in the late afternoon between 3 and 5 p.m. Their food was either tossed in from the tower or side of the tank or through a food delivery tube with running seawater. The person feeding the fish usually hid from the fish, and the place of feeding was randomized. In general, the fish were healthy and ate well throughout the tank tests. Other details of the facilities and general procedures for the maintenance of pelagic tuna can be found in Nakamura (1972).

The typical experimental day began with the placing of the video camera on the roof of the aeration tower overlooking the tank. The video tape recorder and a small B/W video monitor was set up in the tower and the odor delivery system was turned on for the remainder of the day. A fish was usually done in the large tank first, then one in the small tank. The testing was usually done from 10:30 a.m. to 2:30 p.m., when visibility and contrast in the tanks was good.

In general, each test consisted of a 5-min control period of observation before the odor was introduced, and a 5-min odor test period. The odor delivery was begun with sufficient lead time so that the odor reached the tank at the beginning of the test period. The lead time varied depending upon which tank, which station, and which flow rate of the odor were used. This was determined with dye tests. The odor was continuously injected into the delivery system with a syringe pump at a constant rate (either 74.1 ml/min or 114 ml/min).

This procedure resulted in a plume of odor in the tank which slowly dispersed as it moved away from the input station. The plume maintained its shape very well due to the consistent current. Thus, a good approximation of the encounters of the fish with the odor plume could be made from video tape analysis and the dye tests.

The behavior of the fish was observed from the aeration tower while making simultaneous video and audio records with the video tape recorder. Verbal descriptions of behavior, time signals, and operations were recorded, with a hand-held microphone on the audio track.

The normal behavior of the fish in the control period consisted of a slow lapping of the tank (see Nakamura 1962 for other behavioral details). In the large tank, the yellowfin tuna seldom deviated from a slow counterclockwise lapping unless disturbed, feeding, or responding to an odor. The fish in the small tank were more variable in behavior, often mixing "figure-8" and half-lap patterns with the normal lapping pattern. Normally, the fish maintained a close school formation, with an occasional individual breaking away in a burst, half lap or figure 8. During control behavior, swim speeds showed little apparent variation; the normal pattern was a slow cruise, at about 2-3 body lengths/sec. There was little orientation to current by the fish in the small tank, but the fish in the large tank consistently swam against the current. If there was a disturbance of the water surface or if a person stood at the edge of the tank, the fish would break formation and approach to investigate.

The video tapes were analyzed by playback from the video tape recorder on a large video monitor. A clear vinyl overlay, with the tank outline, and estimated position at regular intervals, of the plume (from dye tests), was placed on the monitor screen and superimposed on the video image of the tank

and fish. Appropriate overlays were made for all combinations of tank and station. The dominant swimming pattern and behavioral events for each 15-sec period during the control and the test periods were recorded on special data sheets designed for that purpose. Estimated odor encounter times were also recorded.

The behavior of the fish was then compared between the control and test periods; notable differences indicated that a response had taken place. Mainly differences in swimming patterns in relation to the odor plume and behavioral events were noted. Since the large tank held a school of fish, the dominant behavior of the school was recorded, with notes on unusual behaviors by individual fish. The fish in the small tank, since there were only two or three at a time, were observed individually.

The criteria used in judging a behavioral response were 1) a change in overall swimming pattern if it occurred within about 10 sec of estimated odor encounter and if the change was not seen regularly in the control period, 2) a sudden behavioral response (such as a burst or stall, mouth snapping, or twitch), if it occurred within about 10 sec of odor encounter, 3) a noticeable, significant change in swimming speed within 10 sec of odor encounter, 4) appearance of overt behavioral responses, such as feeding bars, searching, chasing, striking, etc.

The responses were ranked on a scale of 0-5, where 0 = no detectable response, 1 = possible response, 2 = weak response, 3 = a definite, mild response, 4 = strong response, 5 = strong response with overt food search behaviors. (See Table 1 for odors and responses.) To calculate the mean response rank for each odor, the response ranks of all the tests of that odor were summed and divided by the number of tests.

The behavior patterns are defined as follows.

Swimming Patterns:

Lap - A 360° circle of the tank, with no straight line portions contained within. Variable in size, covers at least four quadrants, but similar in form.

Half-lap - A half-circle pattern with a straight line portion connecting the ends of the semicircle. Characterized by sharp-angle abrupt turns at the semi-circle ends. Covers at least two quadrants of the tank.

Loop - A tight 360° circle within one quadrant of the tank.

Turnaround - A tight 180° turn so that the fish reverses its original direction of swimming. Usually within one quadrant.

Figure 8 - Two connecting turnarounds such that a "figure-8" pattern is inscribed in the tank. It can be highly variable, sometimes occurring along the side of the tank, across the center or so large that it appears as two contiguous half-laps.

Speed-up - A noticeably significant increase in swimming speed. Less dramatic and more sustained than a burst. Duration about 15 sec or more.

Search - A slow side-to-side swimming motion, with slow forward progress, usually with pectoral and axial fins spread. Usually head down with a weaving motion, upstream.

Upstream - The fish is swimming upcurrent, into the odor plume.

Downstream - The fish is swimming downcurrent, away from the odor plume.

Behavioral Events:

Burst - A sudden, dramatic increase in swimming speed. Short-term phenomenon. Characterized by an abrupt burst of high speed tail beats, followed by a glide with all retractable fins folded, using just the caudal and a pectoral fin to effect a turn (in a round tank). Usually the individual far outdistances the rest of the school but rejoins it quickly. Duration usually less than 5 sec.

Stall - A momentary cessation of caudal fin movement, resulting in a glide with pectoral fins spread. Often associated with a "yawn" and a "fin-spread."

Yawn - An opening wide of the jaws, usually with a "fin-spread", opercular flaring, and a glide.

Twitch - A sudden, side-to-side shake of the head, with usually one flexure of the body. Not associated with feeding process.

Flash - Fish leans on its side to display the silvery, reflective coloration on the ventral/lateral portion of body. Thought to be an agonistic display.

Feeding Bars - A coloration change due to increased "excitement" during feeding, in anticipation of feeding, or seen in cases where the fish is stressed, frightened, or startled. It manifests itself in various ways in different tunas, but in the yellowfin tuna, "bars" appear as light-colored vertical bars on the body, alternately thin and long and short and thick, covering the entire side of the body aft of the opercles. The body also darkens in background color, so that the

normal dark-medium blue of the dorsal/lateral surface becomes blue-black and a demarcation appears on the dorsal surface of the head behind the opercles. A variation of this pattern is sometimes seen on dead fish, but the bars seem to break up into dashed lines.

Surface Strike - Fish strikes at an object at the surface.

Chase - Fish chases object(s) in the tank, often small fish that are usually ignored.

Snap - A rapid closing and opening of jaws.

Fin-Spread - Simultaneous extension of the pectoral fins and all axial fins. Usually only at slow swimming speeds.

Results

As it can be seen from Table 1, the most effective attractant was squid extract with the addition of tryptophan and alanine. Whole squid extract and synthetic squid and surf smelt were marginally less effective, and surf smelt rinse, freeze-dried surf smelt rinse, and proteinaceous surf smelt extracts were slightly less effective than the former.

The squid extracts also gave a number of "5" responses, which indicates a high degree of arousal. The whole squid extract, including the ink sacs, gave very good responses. Since the commercial California squid is available in large quantity at a moderate price, an augmented extract of squid was arbitrarily chosen for use as the standard attractant in Phase 2 field testing.

The constituents of food odors used as standards are listed in Table 2.

Discussion

As in the previous study, proteinaceous substances seem to be the active substances in most of the prey odors we tested. The lipid fractions generally had a lower level of activity than the nonlipid substances. But because we made our extractions in water, lipids were not extracted as well as the water soluble amino acids.

It has been found that whole-food extracts elicit a stronger response than synthetic mixtures in many higher animals, and in many cases, there seems to be a "fingerprint" odor that is quite complex and is required to elicit the full response (Bardach 1975). In the previous study (Atema et al. 1980) and in this project, such responses seems to be occurring. However, addition of some amino acids approximating a fish rinse to a squid extract seems to have elicited a stronger response. Perhaps it is possible to fabricate a "superodor" that incorporates the most attractive elements of several odors.

PHASE 2 - PRELIMINARY FIELD TESTS

Introduction

This phase of the project was designed to field test the most promising attractants in Hawaiian waters. Since no purse seine vessel operates out of Honolulu, a trolling boat was selected as the test vessel. It was hoped that trolling would yield rapid quantifiable results. Due to the fact that yellowfin tuna are often caught by trolling off the west coast of Oahu, this area was chosen as the study area (Figure 1).

It was decided to use paired tests, trolling in areas where tuna were known to have been caught, alternately with and without laying an odor trail behind the boat.

Methods and Materials

A 44-ft fishing boat, the Kamalii Kai Too, equipped with a scanning sonar, depth recorder, and a loran C receiver was chartered. Operations were based in Pokai Bay on the Waianae coast of Oahu. Fifteen day-trips were made in late May and early June 1978.

Each test began with the dropping of a small flag buoy with a small sea anchor, marking the starting point of the test runs. We then moved away from the flag, trolling seven lines with artificial lures, at about 8 knots for about 10 min along a straight track over a preselected depth. At 10 min elapsed time, we turned 180° and retraced the track back to the flag. If the flag has been displaced by the current, we retrieved it and replaced it at the original starting point. We made the "wet" run on the same track as the "dry" run but pumping odor behind the boat. We usually made one dry run and two wet runs, each including one "away" leg and one return leg. At the conclusion of the wet runs, we picked up the flag and proceeded to another area for a new test series. Thus, except for the presence of the odor trail, the two runs were essentially identical. The flag was used to help account for any current drift on the odor trail.

Any strikes, hook-ups, and landings of fish were entered as data points for their respective runs. If possible, we noted the species and behavior of the fish and observations by the captain and the crewman. Often, strikes would occur "in the blind" unexpectedly while traveling to test areas, before tests could begin or some time after an odor test was run.

The odor was prepared fresh for each test in a 100-liter plastic barrel. The squid for the extract was diced, frozen, and stored in an ice chest on the boat during the tests. The diced squid was put in a nylon netting bag which was dropped into the barrel of seawater, then the amino acids were added. When

the run was finished, the barrel was emptied, the squid disposed of in a bucket, and the apparatus was washed.

The odor trail was delivered by a 1/4 in. i.d. polyethylene tube with a weighted end trailing behind the boat about 3 m. The other end was attached to a small, 110 VAC, submersible pump which was immersed in the barrel. Depending upon the exact configuration of the delivery tubing, the flow rate was about 2 liter/min. Judging from dye tests, the odor trailed in a narrow line behind the boat, mixing with the wake but still leaving a sharp gradient on either side. In some of the later tests, we used both the surface and a subsurface odor release system. The surface system was unchanged, but an additional clear vinyl hose was attached to a downrigger line which took the end of the hose down to a depth of about 5 m. Because of the extra drag of the subsurface gear, we trolled at a lower speed to reduce the chance of breakage. The flow rate for the combined system was about 5 liter/min. Usually one leg was run with both lines, then three more legs were run with only the surface line.

Results

As it can be seen from Tables 3 and 4, the results were highly variable. In some cases, the runs with no odor trail produced more strikes per hour than those with odor. Blind strikes also occurred, due to the inherent attractiveness of the boat and the trolling lures.

Since the strikes tended to be episodic and low frequency in usually productive situations (bird flocks, porpoise schools), it seemed pointless to do a statistical analysis.

Discussion

The results of the trolling tests were disappointing. The tests took place in what was evidently a slack period in productivity and several weeks later, a large number of tuna began appearing in the Waianae area. Perhaps if the tests had taken place during a more productive time, the results would have been more precise.

On May 12, four strikes occurred in a "wet" run in comparison to one strike in the previous "dry" run.

On June 9, when yellowfin tuna were encountered in abundance, five of six large tuna struck while odor trails were laid.

These episodes were the only trials where odors seemed to enhance catch rates. However, the rest of the data do not support such a claim. Although the trolling tests did not indicate whether odors were effective attractants, a squid extract with added amino acids was arbitrarily chosen as the olfactory attractant for the purse seine field tests. Phase 1 testing had proven the effectiveness of this combination.

PHASE 3 - PURSE SEINE FIELD TESTS

Introduction

In May 1978, preparations were begun for the purse seine field tests scheduled for late July. A freeze-dried squid extract with the addition of an amino acid mixture to be dissolved just before use was chosen as the attractant. A whole extract had proven to work better than a fractionated extract, so a synthetic extract was not formulated. Behavioral tests had shown that adding specific amino acids to the squid extract might be beneficial and increase the attractability of the odor.

Methods

Oregon Freeze-Dry Foods, Inc.,[1] was contracted to produce the freeze-dried squid extract. Three hundred kilograms of squid was minced and extracted in 2,835 liters of fresh water for 30 min at a temperature of 7.2°C. The squid was then filtered out and the extract frozen for drying. The extract was freeze-dried at a sublimation temperature of about 38°C. A total of 22.1 kg of freeze-dried extract were repackaged in 30 plastic bags of 735 g each. Each bag contained enough extract to mix 189 liters (50 gal) of attractant. The amino acids were weighed and packed in Honolulu and each sealed package contained:

16.5 g L-glycine	All chemicals from:
9.1 g L-valine	Grand Island Biological Co. 519 Aldo Ave.
8.0 g L-leucine	Santa Clara, CA 95050
3.0 g L-lysine	(see footnote 1)
2.25 g L-tyrosine	
2.0 g L-histidine	
1.0 g L-isoleucine	

This was sufficient for 189 liters. The constituents were selected to approximate a combination of squid and fish extracts. Enough freeze-dried squid and amino acids were brought to enable us to perform 15 full attractant tests, at 378 liters (100 gal) of attractant per test.

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[1]Mention of trade and company names does not imply endorsement by the University of Hawaii or the National Marine Fisheries Service, NOAA.

We performed attractant tests before backdown, through backdown, and during backdown. Pre-backdown tests were begun about 20 min before backdown, soon after "rings-up." Through backdown tests began about 5 min before and continued about 15 min into backdown. During backdown tests began at the commencement of backdown and continued until the end of backdown or until the attractant was exhausted. We were able to pump about 378 liters of attractant in 20 min, a flow rate of about 18.9 liters/min (5 gal/min).

We performed a total of 12 tests, of which 2 were dye-dispersal tests and 10 were attractant tests. Concentrated fluorescein sodium dye was used in the dye tests to ascertain current and plume-spreading characteristics. Five tests were made pre-backdown, three through backdown, and two tests during backdown.

We had a total of 17 sets in 30 days at sea, catching an estimated 49 tons of tuna capturing and releasing an estimated 5,688 porpoise.

The criteria used in judging a response were based on the behavioral studies of Phase 1. Briefly, a positive response was indicated when a fish displayed the following swimming patterns and behaviors.

1. Display of feeding bars - feeding coloration change in anticipation of feeding when fish are in the vicinity of the odor cloud.
2. Dorsal fin erection, pectoral fin spreads, gaping or snapping of jaws.
3. A sudden increase in swimming speed by a rapid increase in tail beat rate or a decrease in speed by a momentary cessation of tail beat.
4. An increased frequency of turning or sharp, abrupt turning.
5. Gradient searching behavior, indicated by a slow, exaggerated side to side movement (weaving motion) oriented upcurrent toward the odor source.
6. A general increase in activity, apparent arousal, or orientation unexplainable by other causes.

7. A breakdown in uniform school swimming pattern resulted in individual swimming patterns and irregular swimming compared to normal school behavior.
8. A localization of the odor cloud indicated by a lengthy stay in the vicinity of the odor plume and a display of the above behaviors after passing through the odor plume or in it.

The appearance of these behavior patterns, especially in relation to No. 8, indicates a positive response. The responses tend to be variable and some fish will display some and not others at a particular time.

For at-sea operating procedures, results, and conclusion of Phase 3, please refer to Bratten et al. 1979.

In summary, the olfactory attractant was judged not effective in separating tuna from porpoise in the purse seine net. Although some localization responses from the tuna were seen, the attractant was not strong enough to break the apparent bond of the tuna and porpoise schools. The olfactory attractant is expected to be useful in alternative fishing methods.

PHASE 4 - ELECTROPHYSIOLOGICAL ASSAYS OF RESPONSES

Introduction

In September 1978, the project was extended for an additional 7 mo to test the feasibility of electrophysiological bioassays of olfactory attractants. Similar techniques had been used with freshwater fish, such as coho salmon, Oncorhynchus kisutch (Dizon et al. 1973), rainbow trout, Salmo gairdneri (Hara 1973), char, S. alpinus (Doving et al. 1974), and some marine species such as searobins, Prionotus carolinus, and squirrel hake, Urophycis chuss (Bardach and Case 1965), and goatfish, Parupeneus porphyreus (Holland 1978).

Methods

Briefly, the general procedure was to immobilize the fish, anesthetize it and keep it alive, expose the organs of interest, and observe bioelectric phenomena with electrodes, preamplifiers and oscilloscopes, while stimulating the olfactory organ with an odor.

Skipjack tuna were used for most of the trials because there were more available and they were less costly than the yellowfin tuna. Yellowfin tuna were used when they became available and when supplies were adequate.

The initial problem was the immobilization of the animal. Tuna are very strong and active fish but extremely fragile. They also depend on ram-gill ventilation (Roberts 1978) and have little opercular pumping ability. They will soon die if held immobile or out of water for a few minutes. The procedure was as follows. A fish was dipnetted from the holding tank and given an injection of Gallamine triethiodide (a neuromuscular blocking agent) in the dorsal musculature at an estimated dose of 2 mg/kg fish body weight. It was immediately released into an adjacent tank where it was allowed to swim freely until its swimming became impaired. Then it was dipnetted out and carried into the laboratory where it was placed into a flow-through perfusion trough on a water table. A hose with flowing aerated seawater (8.3 liters/min, 23.5°C) was placed in the mouth of the fish for respiration; the eyes were covered to reduce struggling, and the spinal cord was cut just behind the cranium to extinguish tail beat movements. Two fine silver-coated wire electrodes were placed in the ventral area over the heart for monitoring the electrocardiogram (ECG) of the fish. The fish was then strapped firmly into the trough and padded with foam blocks to prevent shifting.

Incisions were made in the top of the head, exposing the top of the cranium from just behind the eyes to just behind the posterior nares. The cranial bone was cut and removed with rongeurs and the underlying membranes and fatty tissues were removed, exposing the paired olfactory nerves which run between the eyeballs from the olfactory bulbs under the front of the forebrain to the olfactory rosettes under the anterior nares. The whole nerve was not exposed; only about 1 cm was usually exposed. An aspirator proved necessary during dissection because of the large amount of oozing fluid and blood contaminating the work field. The blood pressure of these fishes is quite high (Tota 1978) and resulted in profuse bleeding from even the smallest arteries. Fortunately, clotting was usually quick.

The paired olfactory nerves were contained in a connective tissue sheath which had to be removed. Care had to be exercised because a large blood vessel ran inside the sheath. Depending upon the type of electrodes being used, the nerves were separated slightly, their nerve sheaths stripped, and the fiber bundles were teased apart for pickup by recording electrodes. During dissection, marine fish saline was used to wash and moisten the exposed tissues. If the bulbs were being recorded, then the same dissection would be performed but centered over the front of the brain about 5 mm behind the eyes. It is my experience that the dissection for the olfactory bulbs is simpler and faster than for the nerves.

In the skipjack tuna and the yellowfin tuna, it was difficult to insert a stimulus cannula into the anterior nares, under which the olfactory rosette lies (Gooding 1963; Iwai and Nakamura 1964). Therefore, the narial region was opened, exposing the rosette so that a cannula to perfuse the rosette continuously and an odor stimulus could be applied. The cannulae were connected to a source manifold of continuously flowing aerated seawater from the same source

as the gill perfusion water. Two glass pipettes, connected to three-way valves and enclosed in water jackets, contained the odor stimuli so that odors at the same temperature and at a similar flow rate to the perfusion water could be directed through the annulae. The manifold was designed to deliver either one of two odors or seawater, at a rate of approximately 0.5 ml/sec, to both nares at the same time.

Several types of recording electrodes were used, including suction, silver hook bipolar and monopolar, platinum hook bipolar and monopolar, tungsten microelectrodes and silver-coated wire for ECG recording.

The suction and hook electrodes were led into a Grass P15B AC differential preamplifier, than a Tektronix 502A dual trace cathode ray oscilloscope and a Brush 220 chart recorder (see footnote 1). An output was also fed to an RC integrating circuit with rectification (time constant = 0.5 sec) and to a separate channel on the 502A and the Brush recorder. An audio monitor fed from the preamplifier completed the set up.

The tungsten microelectrodes used in attempted recordings from the olfactory bulb were amplified by a miniature AC preamplifier and then into the same recording chain described previously.

After unsuccessfully trying to get consistent nerve recordings, the feasibility of cardiac rate deceleration (CRD) as an indicator of a sensory response was explored. In response to thermal stimuli, conditioned skipjack tuna had showed momentary decelerations of cardiac rate (Dizon et al. 1974, 1976). Goldfish have shown cardiac responses to auditory stimuli (Popper, Department of Zoology, University of Hawaii, Honolulu, pers. commun.), and salmon to olfactory stimuli (Hirsch 1977). It was possible that the yellowfin tuna might show a similar response to olfactory stimuli, especially if they were also conditioned.

Using the ECG monitor; electrodes amplified by a Tektronix 122 AC differential preamplifier, a Tektronix 565 oscilloscope, Brush 220 recorder, and an audio monitor, I could see momentary cardiac rate decelerations if they occurred. An RC integrator circuit aided in the visualization of a response. The only dissection required for this procedure was the opening of the narial cavity.

Some fishes were classically conditioned by shutting off the gill perfusion water when an odor was presented. Doing so caused the fish to respond with a CRD. This conditioning (negative reinforcement) was repeated several times and then the fish was tested with odor alone. The fish would usually respond to the odor with a CRD, but the response was extinguished within a short period of time if not reinforced again.

Results

Consistent electrophysiological recordings from the olfactory nerves or the bulbs could not be obtained. Occasional responses were seen that appeared to be genuine nervous activity but these could not be repeatedly obtained.

Cardiac rate decelerations were seen in response to the shut-off of gill-perfusion water, touching of the fish's body, and some odors. However, occasional CRD were also seen in undisturbed fish, often occurring almost periodically in some fish. The CRD responses also tended to extinguish quickly unless the conditioning was repeated.

Because of the lack of a consistent body of data and the approaching end of the contract, the experiments were terminated.

Discussion

The experiments were plaqued by a variety of artifacts, including radio frequency interference from nearby transmission towers, electrical supply problems, and occasional water supply failures. Although there have been successful recordings from the visual system of tuna (Tamura et al. 1972; Hanyu et al. 1973), no successful recordings from the olfactory system have yet been made. The surgical difficulties, high blood pressure thus excessive bleeding, and the fragility of the fish certainly result in a difficult preparation.

The tuna olfactory system will certainly yield successful recordings eventually. However, the preparation was neither reliable nor easy enough to be considered feasible as a rapid bioassy for olfactory attractants at this time. There also remains the fundamental ambiguity of such electrophysiological data. There is no discrimination between attractive and aversive substances. Either one will generate an electrical response from primary sensory cells. It is at the higher nervous centers that integration and discrimination take place. To determine the attractiveness or aversiveness of some odor, behavioral bioassays must still be performed. Once the behavior is known, then electrophysiological methods can be used to obtain rapid threshold and amplitude responses to specific substances.

SUMMARY

1. The most effective odor in captive tuna behavioral tests was a squid extract with amino acid additives, followed by whole squid extract and a synthetic fish and squid mixture of amino acids.
2. Field tests from trolling boats off the Waianae coast of Oahu, Hawaii yielded inconclusive results.
3. In purse seine field tests, an attractant consisting of a freeze-dried squid extract with an amino acid mixture of L-glycine, L-valine, L-leucine, and others, was not effective in breaking the bond between tuna and porpoises in the net. Some positive responses to the odor were shown by some of the tuna. Attractant was judged not effective in the reduction of incidental porpoise mortality.
4. Electrophysiological recordings from the olfactory nerves, and olfactory bulbs of tuna were attempted. Since problems were encountered in obtaining a consistent recording in response to olfactory stimulation, these techniques were judged infeasible as a quick bioassy of attractive odors.

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Table 1.--Odor and test summary.

Odors	Rank	No. tests	No. 5's	Source[1]
Nehu (*Stolephorus purpureus*)				
20 ml test	1.5	2	0	1A
Rinse	1.9	4	1	1A
Surf smelt (*Hypomesus pretiosus*)				
Rinse	2.9	7	0	1
Augmented	2.7	6	2	2
Freeze-dried	3.0	6	1	3
Lipid	1.0	1	0	4
Lipid A	1.0	1	0	4
Lipid B	2.0	1	0	4
Nonlipid	3.0	1	0	5
FW	1.5	1	0	6
Hydro. HiMW	3.0	1	0	7
Synthetic	2.3	3	0	8
Squid (*Symplectoteuthis oualaniensis*)				
Rinse	1.5	6	0	10
Augmented	0.7	3	0	9
Squid (*Loligo opalescens*)				
Rinse	2.1	5	1	10
Augmented	3.7	3	2	9
Nonlipid	0.8	2	0	5
Amino	2.0	1	0	11
Ink extract	0.9	2	0	12
Whole	3.5	2	1	13
Synthetic	2.2	3	0	14
Anchovy (*Engraulis mordax*)				
Rinse	0.5	3	0	1
Shad (*Dorosoma petenense*)				
Rinse	0.5	1	0	1
Flyingfish (*Parexocoetus brachypterus*)				
Rinse	2.0	2	0	1
Dye + WB rinse	410	1	0	15
Synthetic squid + WB	3.5	1	0	16
Seawater	0.5	1	0	

[1]Sources:

1. Standard rinses - Prepared by gently swirling about 200 g of undamaged, fresh dead or fresh frozen fish or animal in 1 liter of filtered seawater for 15 min. Then filtered and the extract stored frozen in glass. Thawed and diluted to about 100 µg/ml before use (if necessary).

1A. Live nehu rinse prepared by dropping an average of 330 g of live nehu dip netted from a tank into 2 liters of filtered seawater. Nehu soaked and gently stirred for 20 min then extract filtered and frozen. Thawed for use.

2. Augmented surf smelt made by adding 180 mg L-tryptophan and 20 mg L-alanine (per liter) to standard surf smelt rinse.

3. Freeze-dried extract initially prepared in standard fashion with distilled water or seawater. Concentrated in a rotary vacuum evaporator ("Rotovac"), then freeze-dried in a small laboratory freeze-drying unit. All FD WB was eventually prepared in distilled water and reconstituted in seawater.

4. Lipid extracts prepared by "Rotovacing" standard WB to dryness, extracting with chloroform, then evaporating the chloroform fraction to dryness and reconstituting with seawater. "Lipids A" is the reconstituted chloroform fraction, and "Lipids B" is the reconstituted residue remaining after the initial chloroform extraction.

5. Nonlipid fraction is the fraction remaining as residue after a standard rinse has been "Rotovaced" to dryness and reconstituted to original volume.

6. Standard WB rinse made with distilled water and sea salts added to the proper salinity.

7. Standard WB rinse which was processed through an Amicon UM-2 ultrafilter. The residue remaining on the filter was reconstituted to a concentration of 14.9 µg/ml, hydrolyzed with 6N HCl for 26 h at 100°C, HCl removed, neutralized and reconstituted to 200 ml with seawater.
8. An amino acid mixture of 10 g each of L-tryptophan, L-leucine, L-valine; 3 g each of L-tyrosine, L-alanine, L-glycine, and Trimethylamine HCl; and 1 g L-glutamic acid in 1 liter seawater.
9. Augmented squid is standard squid rinse plus 920 mg L-tryptophan, and 20 mg L-alanine per liter.
10. Standard squid rinses made the same way as other standard rinses except that the squid was dissected to remove ink sacs, then minced into 1 cm wide pieces for soaking. Symplectoteuthis oualaniensis is the wild, pelagic squid caught in Hawaiian waters, and Loligo opalenscens is the commercial California squid caught offshore. The latter was used because the pelagic squid was almost impossible to get fresh in any quantity and the California squid was readily available in large quantities.
11. Standard squid rinse processed through a Dowex 50 H+ ion exchange column. Fraction that was retained.
12. Intact ink sacs of squid ground in a mortar and pestle with seawater. Filtered and diluted before use.
13. Prepared exactly like standard squid rinse but without having the ink sacs removed, i.e., whole.
14. An amino acid mixture consisting of 10 g each of L-proline, L-alanine, L-betaine; 3 g each of L-glycine, L-taurine, L-leucine, L-tryptophan, and Trimethylamine HCl; and 1 g each of L-aspartic acid, L-lysine, L-glutamic acid; in 1 liter of filtered seawater.
15. Fluorescein sodium dye and standard WB rinse.
16. Synthetic squid + WB is the two synthetic mixtures (8, 14) combined.

Table 2.--Composition of food odors.

Odor	Amino nitrogen (µg/ml) (Moore and Stein 1948)	Principal (in order) amino acids
Live nehu rinse	36	Gly, Ala, Lys, Val, Leu, Ser.
Standard WB rinse	270	Gly, Ala, Val, Leu, Glu, Ser.
Standard squid rinse	1,200	Pro, Ala, Gly, Leu, Val, Ser, Glu.
Anchovy	190	Try, Leu, Val, Lys, Tyr, Ala, Gly, His, Thr.
Flyingfish	71	Ala, Leu, Val, Pro, Arg, Phe, Gly.

Amino acid composition obtained from TLC chromatographic analysis and gas chromatograph.

Ninhydrin concentration of amino nitrogen by spectrophotometric analysis by modified Moore and Stein (1948) method, standards prepared in salt water.

Table 3.--Phase 2 - Field test summary.

Date 1978	Elapsed time (min)	Odor	Amount (per 80 liters)	No. of strikes and species[1]	Location and comments
May 8	120	No	--	1 DO	On 1,000-fathom ledge, in birds
	160	Yes	2 kg squid	1?	On 1,000-fathom ledge
May 9	20	No	--	--	In bird flock
	50	Yes	Same odor	--	Same area
	30	No	--	--	In dolphin school
	25	Yes	Same odor	--	In same area
	45	No	--	1 spearfish	Blind strike
	80	Yes	Same odor	1 WA	Likely to have smelled odor
May 10	20	No	--	1 YF	120-fathom line, one blind strike previously
	35	Yes	Same odor	1?	Same area
	45	Yes	Same odor	1 SJ, 1?	Same area, N-S direction
	150	No	--	1 YF, 1 SJ, 1?	In bird flock
	160	Yes	Same odor	1 SJ	In skipjack tuna school, 1,000 fathoms
	140	No	--	2 SJ	Same area
	30	No	--	--	100-fathom deep
	35	Yes	Same odor	--	Same area
May 11	60	No	--	--	1,000 fathoms, flyingfish seen
	40	Yes	Same odor	--	Same area
	35	Yes	Same odor	--	Same area, opposite direction
	35	Yes	Same odor	--	1,000 fathoms, different area
	30	No	--	2 SK	In bird flock
	25	No	--	--	100-fathom line
	30	Yes		--	Same area

Table 3.--Continued.

Date 1978	Elapsed time (min)	Odor	Amount (per 80 liters)	No. of strikes and species[1]	Location and comments
May 12	45	No	--	--	100 fathom, NE direction
	31	Yes	2 kg squid	--	Same area
	45	No	--	1 DO	40 fathoms, blind strike
	50	Yes	2 kg squid + amino acid[2]	2 WA, 1 YF, 1?	Same area, within 5 min.
	40	No	--		Same area
	52	Yes	2 kg squid	--	Same area
May 22	34	No	--	--	1,000 fathoms, with flag
	60	Yes	2 kg squid + 24 g amino acid[3]	--	Same area
	30	No	--	--	1,000 fathoms, opposite heading
	50	Yes	2 kg squid + 15 g amino acid[3]	--	Same area
	10	Yes	Same odor	1?	While dumping excess odor
	27	No	--	1? (prob. marlin)	Blind strike
	53	Yes	2 kg squid	--	Same area
	30	No	--	--	100 fathoms
	60	Yes	2 kg squid + 24 g amino acid[3]	--	100 fathoms-150 fathoms
	10	No	--	--	40 fathoms
	45	Yes	2 kg squid	--	Back to Pokai Bay
May 23	20	No	--	--	1,000 fathoms, NW heading
	50	Yes	2 kg squid	--	Same area
	23	No	--	--	100 fathoms
	45	Yes	Same odor	--	Same area
	30	No	--	--	500 fathoms, SE heading
	45	Yes	Same odor	--	Same area
	20	No	--	--	40-fathom line
	57	Yes	Same odor	1?	Same area
				2 WA	Caught 7 min after dumping odor and used squid overboard

Table 3.--Continued.

Date 1978	Elapsed time (min)	Odor	Amount (per 80 liters)	No. of strikes and species[1]	Location and comments
May 24	22	No	--	--	On current interface line
	37	Yes	2 kg squid + 24g amino acid[3]	--	Skipjack tuna school seen in area
	20	No	--	--	500-fathom line, off Nanakuli
	43	Yes	Same odor	--	Same area
	16	No	--	--	In bird flock
	20	Yes	2 kg squid + 24g amino acid[3]	--	Birds seen feeding in wake
	5	No	--	--	In porpoise school
	5	Yes	Same odor	--	Same area
	6	No	--	--	In bird flock
	9	Yes	Same odor	--	Same area
May 25	20	No	--	--	100 fathoms off Makaha
	40	Yes	2 kg squid + 24g amino acid[3]	1 marlin, 1?	6 min after run, same area
	28	No	--	--	1,000 fathoms
	36	Yes	Same odor	--	Same area
	20	No	--	--	500 fathoms, NW heading
	41	Yes	Same odor	--	Same area
	8	No	--	--	In bird flock
	8	Yes	Same odor	--	Same area
	10	Yes	Same odor	--	Same, fish deep
	25	No	--	--	In bird flock
	20	Yes	Same odor	--	Same area
May 26	15	No	--	5? (prob. marlin)	In porpoise school
	10	Yes	2 kg squid + 24g amino acid[3]	--	Same area
	10	Yes	Same odor	--	Same area

Table 3.--Continued.

Date 1978	Elapsed time (min)	Odor	Amount (per 80 liters)	No. of strikes and species[1]	Location and comments
May 26	9	No	--	--	Near flagline set
	24	Yes	Same odor	--	Same area
	20	No	--	--	In area of birds and fish
	27	Yes	2 kg squid + 24 g amino acid[3]	--	Same area
	9	No	--	--	1,000 fathom line
	32	Yes	Same odor	--	Same area
June 9	--	No	--	1 YF	Blind, on way out
	24	No	--	1 WA	Same area
	11	Yes	2 kg squid + 72 g amino acid[4]	--	Same area
	10	No	--	1 YF	Similar area to previous, birds
	5?	Yes	Same odor	3 YD (2 caught)	Same, caught 2 min after odor in
	--	No	--	1 YF	Blind strike 15 min after
	5	No	--	--	Same area previous, 20 min after
	45	Yes	Same odor	1 YF	Same, yellowfin tuna seen following
	--	No	--	1 YF	On way home, had the best catch of any boat that day
June 12	--	No	--	1 WA	Blind strike
	10	No	--	--	In birds and fish
	10	Yes	2 kg squid + 72 g amino acid[4]	--	Same area
	21	Yes	Same odor	--	In same area
June 13	--	No	--	1 marlin (not caught)	Blind strike
	10	No	--	--	In bird flock
	40	No	--	--	In bird flock
	40	Yes	2 kg squid + 72 g amino acid[4]	--	Same area
	--	No	--	1 marlin (not caught)	Blind strike
	30	Yes	Same odor	--	20 min after strike

[1]Species: YF=yellowfin tuna, Thunnus albacares; WA=wahoo, Acanthocybium solandri; DO=dolphinfish, Coryphaena hippurus; SJ=skipjack tuna, Katsuwonus pelamis; spearfish=Tetrapturus angustirostris; ?=unknown.

[2]Squid augmentation solution, 920 mg L-tryptophan, 20 mg L-alanine per liter, 2 liters added to 80 liters seawater.

[3]12 g L-tyrosine and 12 g L-tryptophan, 7.5 g of each.

[4]24 g each of L-tryptophan, L-tyrosine and L-glycine

Table 4.--Catch rate summary.

Treatment	Total time (h)	Number of strikes	Strikes per hour
No odor	14.75	11	0.75
2 kg squid	18.47	9	0.49
No odor	0.75	1	1.33
2 kg squid + amino acid[1]	0.83	4	4.80
No odor	0.50	0	0.00
2 kg squid + 15 g amino acid[2]	0.83	0	0.00
No odor	4.78	5	1.05
2 kg squid + 24 g amino acid[2]	8.20	2	0.24
No odor	1.65	2	1.21
2 kg squid + 72 g amino acid[3]	2.70	4	1.48

[1]Squid augmentation solution, 920 mg L-tryptophan, 20 mg L-alanine per liter, 2 liters added to 80 liters seawater.

[2]12 g L-tyrosine and 12 g L-tryptophan, 7.5 g of each.

[3]24 g each of L-tryptophan, L-tyrosine and L-glycine.

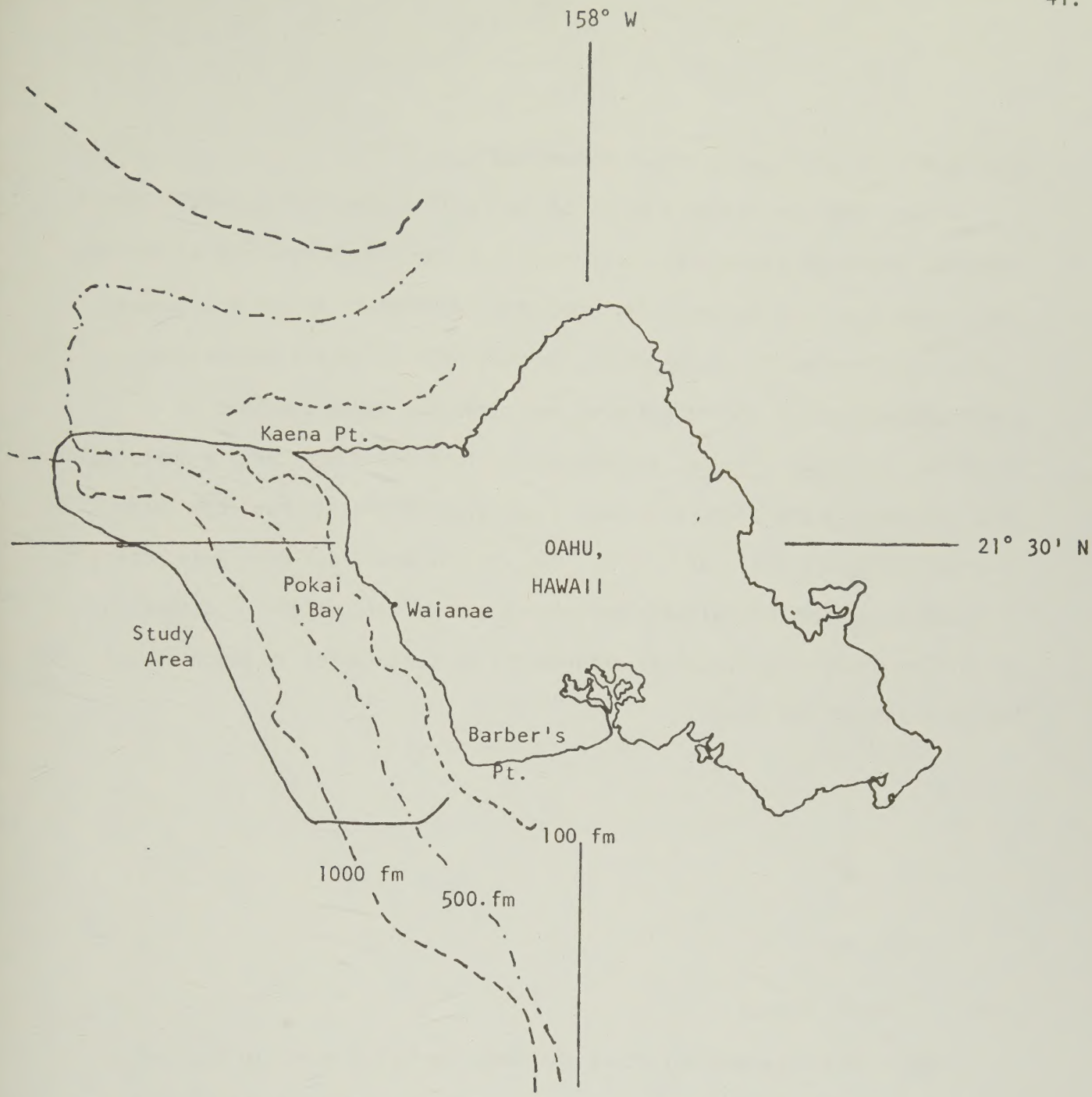

Figure 1. Location of Phase 2 Field Tests

ADDENDUM 1--Electrophysiological Recordings.

In late 1980, Dr. Richard Brill of the Pacific Gamefish Foundation and I obtained electrophysiological recordings from the olfactory bulbs of mullet, Mugil cephalus, thus successfully repeating experiments by Goh and Tamura (1980).* Very recently, in December, we were able to obtain unequivocal electrophysiological recordings from the olfactory nerve and bulb of a kawakawa, Euthynnus affinis, in response to food odors and certain amino acids. This is possibly the first time that successful recordings have been made from the olfactory tract of a tuna. Our success would not have been possible if it were not for the valuable experience and techniques that I gained in my earlier electrophysiological experiments on tuna funded by the National Marine Fisheries Service.

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